

Chemical composition and antimicrobial activity of essential oils in Xerophytic plant *Cotula cinerea* Del (Asteraceae) during two stages of development: flowering and fruiting

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ABSTRACT

The essential oil (EO) of *Cotula cinerea* Del collected in the region of Oued Souf (South-eastern Algeria) during two stages (flowering and fruiting), gave a performance that are $0.0801\% \pm 0.0117\%$ at the flowering stage; and $0.391\% \pm 0.0664\%$ at the fruiting stage. The chemical study of the (EO) of *C. cinerea* Del, analyzed by GCMS, showed the presence of 22 chemical compounds in the flowering period with the dominance of: 3-Carène (30.99%), Thujone (21.73%), Santolina triene (18.58%) and Camphor (6.21%). While 21 chemical compounds were obtained during the fruiting period with the dominance: Thujone (28.78%), 3-Carène (15.90%), Eucalyptol (15.13%), Santolina triene (13.38%) and Camphor (7.49%). Viewpoint chemical kinetics and composition, it was noticed that during the flowering stage the plant has produced eight compounds that are absent during the fruiting period. While seven other compounds appeared only during fruiting. It appears the antibacterial of (EO) has *C. cinerea* Del The sensitivity of the bacterial strains tested ; And *E. faecium*, *E.coli*, *M. morgani*, *P. vulgaris*, *S. aureus* and *A. baumannii* has shown great sensitivity; the strain *P. aeruginosa* have shown stiff resistance with every concentrations of (EO). As we did not notice any differences significant in the diameters of inhibition with all strains in two stages of growth.

INTRODUCTION

Cotula cinerea Del is one of 03 species belonging to the genus *Cotula* (Asteraceae) existing in Algeria [Syn. *Brocchia cinerea* (Del) Vis.] (Dendougui *et al.*, 2012; Maiza *et al.*, 1993). It is a common Saharo-arabic species that grows in the Sahara desert and sandy areas (Quezel and Santa, 1963). Among these regions Oued Souf (Halis, 2007; Ozenda, 1977) where it is locally known as (Shihia/Shihit El Ebel) (Halis, 2007). It is an annual herbaceous woolly plant (Benzizerara *et al.*, 2012) of 5-15 cm completely tomentose. Its stems are erect or diffuse. The

leaves and whitish-green stems are covered with tiny hairs (Quezel and Santa, 1963), thick, velvety small leaves, whole are cut into three to seven teeth or "fingers" (Benhammou, 2012) and in the shaft of high branch yellow inflorescences (Quezel and Santa, 1963; Halis, 2007). This plant contains many chemical compounds with therapeutic benefits such as flavonoids (Dendougui *et al.*, 2012), terpenes and essential oils that give plant odor specificity (Markouk *et al.*, 1999). Leaf extracts are *Cotula cinerea* Del are effective against pathogenic microscopic fungi, they have insecticidal activity on the insect larvae (Bouziane, 2002). This species is widely used in traditional Moroccan medicine for its biological properties such as anti-inflammatory, analgesic, antiseptic, antibacterial, antipyretic (Benhammou, 2012), it can be used to treat stomach pain, fever, headaches, migraines, cough and joint inflammation (Chouikh and Chefrour, 2014).

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The aims of this study highlight the potential fluctuations in yields of essential oils of *Cotula cinerea* Del and chemical composition, and estimate of Antimicrobial activity of essential oils in two phases of growth: flowering and fruiting.

METHODS AND MATERIAL

Plant material

The plant material consists of the aerial part of the plant *Cotula cinerea* Del; harvested in Oued Souf Sahara (South East of Algeria) during two stages (flowering period in February 2010, and fruiting in April 2010).

Extraction of Essential Oils

The plant material (100g MS) is subjected to extraction by steam distillation in a Clevenger-type apparatus (Clevenger, 1928). The distillation was carried out for four hours in accordance with the recommendations of the European Pharmacopoeia (AFNOR, 2000 ; Lmachraa *et al.*, 2014; Velasco-Negueruela and Perez-Alonso, 1990). The extractions were repeated five times to confirm the return earned by the mode used. The essential oil is stored and maintained optimally at 4 ° C protected from light (Moumni *et al.*, 2013a).

According to (Moumni *et al.*, 2013b) the performance of the essential oil, expressed in ml per 100 g of dry matter of *Cotula cinerea* Del, and calculated by the following equation:

$$T_{EO} = \{V/M_S \times 100\} \pm \{\Delta V/M_S \times 100\}.$$

T_{EO} : Performance of the essential oil.

V: volume of essential oil collected (ml).

ΔV : Reading error.

MS: dry plant mass (g).

Chemical characterization of essential oil by GC/MS

The essential oil of *Cotula cinerea* Del was analyzed by chromatography phase gas (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q ion trap MS). The database used for the identification of chemical compounds and measurements of peak areas obtained is that of NIST / EPA / NIH MS LIBRARY (NIST 05) and also AMDIS version 2.0 d.

Antimicrobial activity

Antimicrobial activity of *C. cinerea* essential oils were determined by agar disc diffusion method (Malabadi *et al.*, 2012), Filter paper discs (Whatman N° 3 and 6 mm in diameter) were sterilized by autoclaving (Malabadi *et al.* 2012).

The different concentrations of essential oils (1/1: 100% ; 1/2: 50% ; 1/4: 25% ; 1/8: 12.5% ; 1/16: 6.25% ; 1/32: 3.12%) were easing by DMSO (Di Methyl Sulf Oxide); this concentrations tested against set of pathogenic bacteria: two Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecium* (*E. faecium*), seven Gram-negative bacteria: *Escherichia coli* (*E. coli*), *Morganella morganii* (*M. morganii*), *Citrobacter freundii* (*C. freundii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus vulgaris* (*P. vulgaris*), *Acinetobacter*

baumannii (*A. baumannii*) and (*K. pneumonia*). The pure bacterial strain was obtained from *Laboratories of microbiology, Medical Science Faculty, University of Badji Mokhtar - Annaba, Algeria.*

The plates were evaluated after incubation at 37° C for 24h after which the zones of inhibition around each disc were measured in mm (Malabadi *et al.* 2012).

RESULTS AND DISCUSSION

Organoleptic characteristics of the essential oil extracted:

Essential oils are viscous and persistent odor Pleasant Artémises (Table 1), yellow in flowering and Yellowish Green in fruiting during; the color is strongly influenced by the nature of the complex mixture of oil compounds (Ashnagar *et al.*, 2007).

The yield of essential oils:

The essential oil yield is very low during flowering (0.0801% ± 0.0117%) compared to the fruiting period (0.391% ± 0.0664%) (Fig. 1). It is relatively higher than those obtained by Kether and colleagues (Kether *et al.*, 2012) working on different parts of *Cotula coronopifolia* L. in Tunisia (0.01134% in leaves; 0.03935% for flowers; 0.00123% in the roots and 0.00405% for the rods).

Chemical composition of essential oils of *Cotula cinerea* Del

The results of the analysis by GCMS showed that the essential oil of the flowering period (Table 2) contains 22 chemical compounds whose major constituents are: 3-Carène (30.99%), Thujone (21.73%), Santolina triene (18.58%) and Camphor (6.21%). Other compounds are present with low percentages such as: Eucalyptol (2.79%); 7'-Oxaspiro[cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)]nonan-2'-one] (2.98%); Terpinen-4-ol (3.64%); *p*-Menth-1-en-8-ol (3.01%) and Trans-Pinocarveol (1.28%). The collection of the plant in the fruiting period allowed us to obtain the essential oils containing 21 compound s. The major substances are: Thujone (28.78%); 3-Carène (15.90%), Eucalyptol (15.13%); Santolina triene (13.38%) and Camphor (7.49%). In second position, five compounds are present with average proportions such as: *M*-Cymene (3.34%); 7'-Oxaspiro [cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)] nonan-2'-one] (3.31%); 4(10)-Thujen-3-ol, stereoisomer (1.47%); Terpinen-4-ol (4.26%) and *p*-Menth-1-en-8-ol (1.65%). Other compounds are present in small amounts: Origanene; Pinene; Camphene; beta-Phellandrene; 3-Thujanone; Isoborneol; *p*-Cymen-8-ol and 1,2,2,3-Tetramethylcyclopent-3-enol.

The chemotype obtained during the two periods of plant development is different in regard to chemical compounds and their percentages. The collection of the plant at the flowering stage allowed us to obtain an essential oil dominated by four compounds chapoter by: 3-Carène (30.99%); Camphor (6.21%); Thujone (21.73%) and Santolina triène (18.58%), whereas fruiting stage Thujone prevails with a percentage of (28.78%) followed by 3-Carène (15.90%); Eucalyptol (15.13%); Santolina triene (13.38%) and Camphor (7.49%). The results obtained during fruiting are in

agreement with the data of Tadrent *et al.*, (2014) working on *Cotula cinerea* of Morocco whose chemotype is Thujone (41.4%); and El Bouzidi *et al.*, (2011) whose major compound is the Trans-Thujone (41.4%). Note that in the fruiting period (Figure 02): There is a significant increase in compound s: Eucalyptol; m-Cymene and Thujone; also a non-significant increase of: 3-Thujanone ; 7'-Oxaspiro[cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)]nonan-2'-one] ; Camphor and Terpinen-4-ol. A net decrease in the production of some compounds was observed: 3-Carene; p-Menth-1-en-8-ol and Santolina triene. The same is attributed to:1,2,2,3-Tetramethylcyclopent-3-enol; p-Cymen-8-ol; 2-Isopropenyl-5-methyl-4-hexenyl acetate. With respect to n-Valeric acid cis-3-hexenyl ester, the rate remained the same in both periods.

The plant produced 08 compounds during the flowering period [Trans-Pinocarveol ; Cis-3-Hexenyl Butyrate ; Isobornyl Propanoate ; cis-Piperitol ; Cuminc alcohol ; Carvacrol ; p-Menthane-1,2,3-triol ; Limonen-6-ol, Pivalate]. While 07 new compounds appeared during the fruiting period [4(10)-Thujen-3-ol, stereoisomer ; Origanene ; Pinene ; Camphene ; beta-Phellandrene ; Isoborneol ; (+)-trans-Chrysanthenyl Acetate]. The essential oil of *Cotula cinerea* Del is characterized by changes in the chemotype, due to various factors including for example: the part of the plant used (drugs), stage of plant development (Hammoudi and Hadj Mahammed, 2010), genetic factors (Lmachraa *et al.*, 2014), the environment and the harvest period and the nature of the soil (Fellah *et al.*, 2006).

Table 1: organoleptic characteristics of the essential oil of *Cotula cinerea* Del.

Essential oils of <i>Cotula cinerea</i> Del	organoleptic characteristics			
	Appearance	Color	Odor	Taste
	Flowering	liquid	Yellow	Pleasant Artémises
fruiting	Liquid	Yellowish Green	Pleasant Artémises	Sweet

Table 2: Chemical composition of essential oils of *Cotula cinerea* Del collected in during flowering and fruiting.

flowering					fruiting				
N°	Ret Time	area	area %	compounds	Ret Time	area	area %	compounds	
01	7.211	2868758	0.30	m-Cymene	3.395	127438434	4.22	Santolina triene	
02	7.449	26517689	2.79	Eucalyptol	3.880	6943898	0.23	Origanene	
03	9.011	3383662	0.36	Cis-3-Hexenyl Butyrate	4.034	14288331	0.47	Pinene	
04	10.089	3472222	0.37	3-Thujanone	4.373	11056004	0.37	Camphene	
05	10.663	206241956	21.73	Thujone	5.175	6375286	0.21	.beta.-Phellandrene	
06	10.884	28277266	2.98	7'-Oxaspiro [cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)]nonan-2'-one]	6.936	100935774	3.34	m-Cymene	
07	11.394	58970182	6.21	Camphor	7.247	457541014	15.13	Eucalyptol	
08	11.782	12147362	1.28	Trans-Pinocarveol	9.878	30022904	0.99	3-Thujanone	
09	12.780	7575998	0.80	Isobornyl Propanoate	10.687	869869318	28.78	Thujone	
10	13.167	176332161	18.58	Santolina triene	10.831	100013514	3.31	7'-Oxaspiro[cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)]nonan-2'-one]	
11	13.421	34578305	3.64	Terpinen-4-ol	11.356	226506241	7.49	Camphor	
12	13.551	7814366	0.82	p-Cymen-8-ol	11.689	44462471	1.47	4(10)-Thujen-3-ol, stereoisomer	
13	13.976	28587584	3.01	p-Menth-1-en-8-ol	12.659	18483050	0.61	Isoborneol	
14	14.077	6126622	0.65	1,2,2,3-Tetramethylcyclopent-3-enol	13.155	277084938	9.16	Santolina triene	
15	14.810	5635594	0.59	Cis-Piperitol	13.375	128820727	4.26	Terpinen-4-ol	
16	16.249	5348508	0.56	N-Valeric Acid Cis-3-Hexen-1-Yl Ester	13.490	9205742	0.30	p-Cymen-8-ol	
17	18.215	294131766	30.99	3-Carene	13.926	49927844	1.65	p-Menth-1-en-8-ol	
18	18.296	4549808	0.48	Cuminc alcohol	14.055	7294071	0.24	1,2,2,3-Tetramethylcyclopent-3-enol	
19	18.858	13109553	1.38	2-Isopropenyl-5-methyl-4-hexenyl acetate	16.187	17233248	0.57	n-Valeric acid cis-3-hexenyl ester	
20	19.139	5465117	0.58	Carvacrol	17.210	10620966	0.35	(+)-trans-Chrysanthenyl Acetate	
21	25.607	5103929	0.54	p-Menthane-1,2,3-triol	18.233	480658650	15.90	3-Carène	
22	27.385	8910561	0.94	Limonen-6-ol, pivalate	18.852	24515661	0.81	2-Isopropenyl-5-methyl-4-hexenyl acetate	
Sum			99.58%				99.86%		

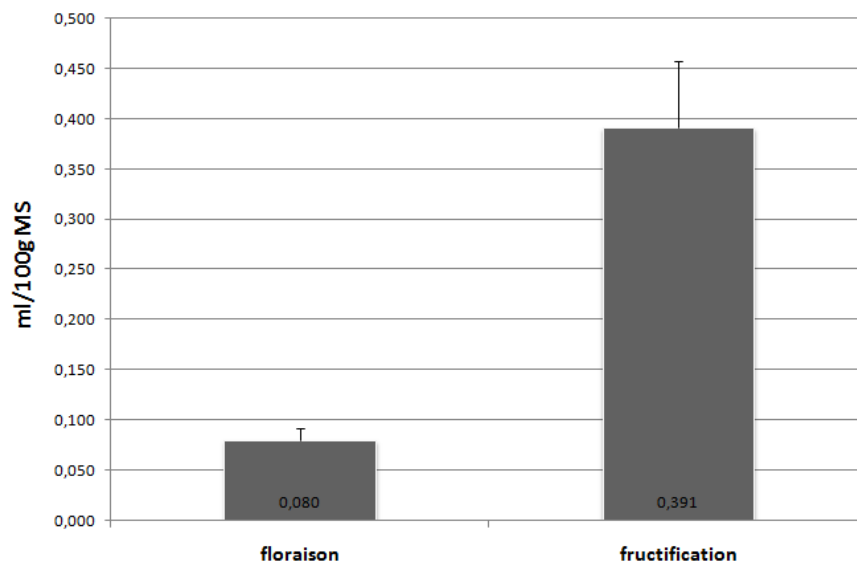


Fig. 1: Yield of essential oils from the aerial part of *Cotula cinerea* Del of Oued Souf region (Algeria).

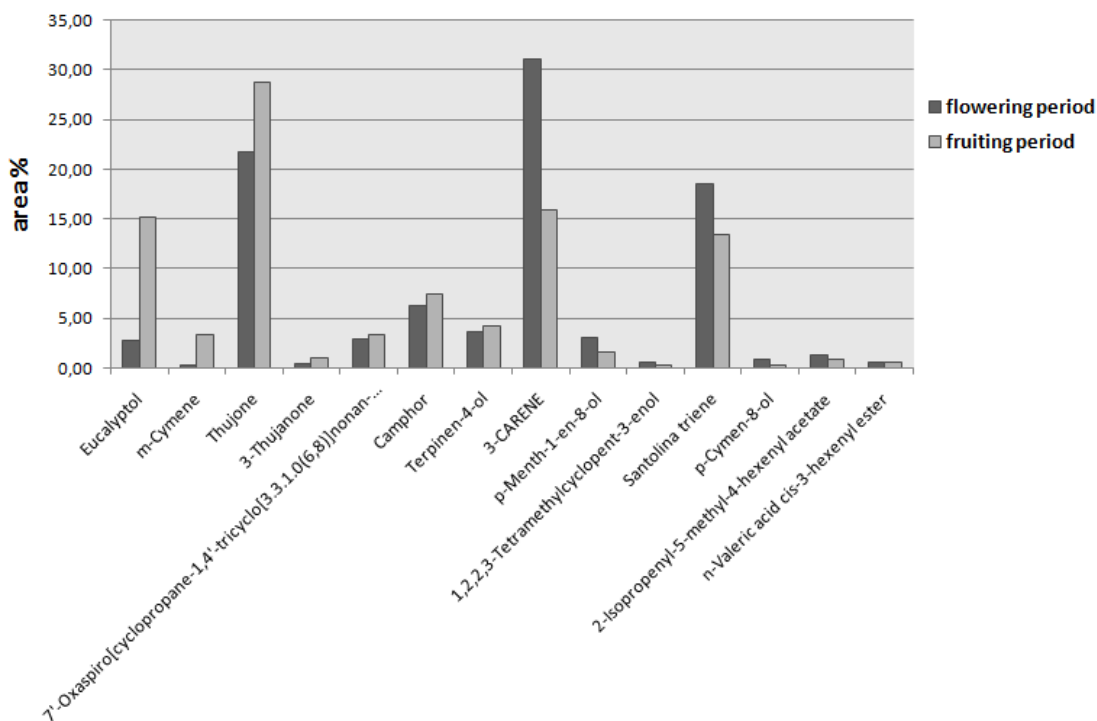


Fig. 2: Kinetics and variation in the chemical composition of the essential oils of *Cotula cinerea* Del obtained during the two stages of development.

Table 3: Inhibition by essential oils of *Cotula cinerea* Del obtained during the two stages of development. (zone size, mm). (IMP: Imipénème), (PEF : Péfloxacine), (L: Lincomycine), (RA: Rifampicine).

Test bacteria	Different concentrations of the essential oils																Antibiotics			
	Flowering period								fruiting period								IMP	PEF	L	RA
	DMSO	Disc empty	1/1	1/2	1/4	1/8	1/16	1/32	DMSO	Disc empty	1/1	1/2	1/4	1/8	1/16	1/32				
<i>E.coli</i>	6	6	50	50	50	50	16	12	6	6	50	50	50	50	18	13	-	-	-	-
<i>M. morgani</i>	6	6	50	50	50	28	16	13	6	6	50	50	45	27	15	10	-	-	-	-
<i>C. freundii</i>	6	6	50	50	21	15	15	11	6	6	50	50	23	19	14	9	-	40	-	-
<i>P. aeruginosa</i>	6	6	18	15	12	10	8	6	6	6	18	9	9	9	7	6	21	06	-	-
<i>P. vulgaris</i>	6	6	47	45	28	21	13	11	6	6	47	46	41	37	18	11	-	-	-	-
<i>E. faecium</i>	6	6	50	50	50	50	50	47	6	6	50	50	50	50	50	50	-	-	32	-
<i>S. aureus</i>	6	6	50	50	50	49	16	12	6	6	50	50	50	50	15	10	-	-	-	-
<i>A. baumannii</i>	6	6	50	50	50	50	18	10	6	6	50	50	50	50	18	10	-	18	-	20
<i>K. pneumonia</i>	6	6	50	50	50	16	15	12	6	6	50	50	50	17	15	12	-	30	-	-

Antimicrobial activity of essential oils of *Cotula cinerea* Del obtained during the two stages of development

Through the results listed in the table (03) conclude that:

- *E. faecium* showed great sensitivity to all with essential oil concentrations where arrived in (50 mm), which was larger than the diameter discourage antibiotic (Lincomycine: 32 mm).
- The strains *E.coli*, *M. morgani*, *P. vulgaris*, *S. aureus* and *A. baumannii* has shown great sensitivity to concentrations (1/1, 1/2, 1/4, 1/8) Where diameter of inhibition ranged between (50 mm to 21 mm), while this strains have shown considerable resistance with concentrations (1/16 and 1/32).
- *C. freundii* and *K. pneumonia* shown great sensitivity to concentrations (1/1, 1/2, 1/4) where diameter of inhibition arrived to 50 mm, as shown resistance medium with concentrations (1/8, 1/16, 1/32) Where ranged diameter of inhibition (16 mm to 11 mm).
- *P. aeruginosa* have shown stiff resistance with every concentrations of essential oil and increased the intensity of this resistance especially with concentrations of essential oil in the fruiting stage.
- Comparing the results of the study of antibacterial of essential oils extracted in two phases: flowering and fruiting, did not record any differences significant in the diameters of inhibition with all strains, except for those registered with *P. aeruginosa* Where showed stiff resistance with all concentrations of essential oil in Phase fruiting compared with concentrations of essential oil in stage of flowering.
- The values of diameters of inhibition were great and arguing with most concentrations compared with diameters of inhibition recorded antibiotics used except antibiotic (Imipénème) with *P. aeruginosa*, which gave diameters of inhibition (21 mm) larger than the diameter suppressive effect of different concentrations of essential oil of flowering and fruiting stages with this strain.

Comparing our results with the results obtained by:

- Bouabdelli *et al.*, (2012) Who experienced several extracts (infusion; decoction; maceration; percolation) on: *E.coli* and *S. aureus* gave diameters small inhibitory Compared diameters obtained where you get to Higher diameter inhibition (20 mm *E.coli* with and 17 mm with *S. aureus*); As with strain *P. aeruginosa* the results were close (14mm).
- Bensizerara *et al.*, (2012) Who experienced different extracts (petroleum ether; ethanol 70%; n-butanol; ethyl acetate) on: *E.coli*; *K. pneumonia* and *S. aureus* gave diameters small inhibitory Compared diameters obtained where you get to Higher diameter inhibition (13 mm *E.coli* with, 12 mm with *S. aureus* and 17 mm

with *K. pneumonia*); As with strain *P. aeruginosa* the results were spaced somewhat (12mm).

CONCLUSION

The chemical study of essential oil of *Cotula cinerea* Del, harvested in Oued Souf region (North Eastern Algerian Sahara), revealed that the optimum essential oil yield obtained during the fruiting period (0.391% ± 0.0664%) is characterized by viscous lingering odor and a yellowish green color while in the flowering period it has a yellow color.

The essential oil of *C. cinerea* Del includes 22 chemicals compounds dominated by: 3-Carène (30.99%), Thujone (21.73%); Santolina triene (18.58%) and Camphor (6.21%), while in the fruit, the number is decreased by 21 compounds of which five are in the majority: Thujone (28.78%); 3-Carène (15.90%); Eucalyptol (15.13%); Santolina triene (13.38%) and Camphor (7.49%). During the fruiting phase the plant produced three compounds in excess (Eucalyptol, m-Cymene and thujone), then the ratio is decreased to three other substances (3-Carène, p-Menth-1-en-8-ol and Santolina triene). the plant synthesized 08 compounds at flowering stage [Trans-Pinocarveol / Cis-3-Hexenyl Butyrate/ Isobornyl Propanoate /cis-Piperitol/Cuminic alcohol / Carvacrol / p-Menthane-1,2,3-triol /Limonen-6-ol, Pivalate], This is probably because these substances have a direct relationship with pollination and fertilization, whereas they are dispensable to the fruiting stage.

According to the results, we can conclude that the yield and chemical composition of essential oils have a relationship with intrinsic (genetic factor, harvest stage, state of the plant) and extrinsic factors (soil, climate, flora procession, insect pests and pollinators, human impact, etc ...). It also appears that the antibacterial essential oil has *Cotula cinerea* Del The sensitivity of the bacterial strains tested by the method of direct contact with the essential oil of *Cotula cinerea* Del varies from strain to strain; And *E. faecium*, *E.coli*, *M. morgani*, *P. vulgaris*, *S. aureus* and *A. baumannii* has shown great sensitivity; the strain *P. aeruginosa* have shown stiff resistance with every concentrations of essential oil.

As we did not notice any differences significant in the diameters of inhibition with all strains in two stages of growth (flowering and fruiting).

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